the oospore is mainly, if not entirely, provided by the generative cell. A resting nucleus is formed.

The oospore elongates towards the top of the endosperm. The first nuclear division within it is followed by the formation of a centripetally developed wall which separates the upper "primary suspensor" from a lower terminal cell. From the latter are developed: (*a*) 24 cells which, surrounding the lower part of the primary suspensor, form with it the "secondary suspensor"; (*b*) a terminal group enclosing a presumed embryonic plate of eight cells. The later stages of embryo-development have not been seen; they possibly occur, as in Gnetum, after the seed is detached from the plant.

It is suggested that (1) The Gnetum-Welwitschia alliance has its origin in the same stock as the angiosperms, but separated from the angiosperm line before the carpel became the pollen-receiver; (2) Welwitschia is the most specialised living representative of the race to which it belongs.

On the Presence of Hæm-agglutinins, Hæm-opsonins, and Hæmolysins in the Blood obtained from Infectious and Non-Infectious Diseases in Man. (Preliminary Report.)

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(Communicated by Prof. T. G. Brodie, F.R.S. Received July 31,—Read November 12, 1908.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

This preliminary report is for the purpose of introducing certain results which have been obtained by allowing normal and immune human serum to act in the presence of normal and immune blood cells.*

These experiments have brought to light many interesting and important points in human hæmatology. The unlimited number of experiments which have been made on immune substances in the blood of the lower animals in comparison to similar investigations in man is most striking. It is necessary to draw special attention to the fact that the work of others has been either briefly referred to or omitted merely because this report is only intended to draw attention to the main facts met with in these investigations, avoiding as far as possible a detailed survey of the subject.

* The expression "immune cell" and "immune serum" is used to designate the blood-cells and sera taken from cases of any general disease or condition. The term "immunity," *i.e.*, is not confined, in this communication, to the series of phenomena resulting from bacterial infection alone.

Nature of Cases investigated.

Every care has been taken to make the classification of the various diseases which have been investigated accurate. Wherever it is permissible, the various groups and sub-groups are arranged as the result of a combination of pathological, bacteriological, and clinical investigations* :—

Group I.—10 cases of acute pneumonia and acute empyæma.

Group II.—22 cases of tuberculosis (mostly acute pulmonary), and 1 case of leprosy.

Group III.—12 cases of epilepsy.

Group IV.—5 cases of acute peritonitis due to appendicitis.

Group V.—5 cases of infections due to the bacilli of the typhoid and paratyphoid family.

Group VI.—26 cases of anæmia, including 14 of pernicious anæmia, chlorosis, lymphæmia, myelæmia, congenital cholæmia, and examples of anæmia secondary to various well recognised conditions.

Group VII.—7 cases of infections due to the streptococci.

Group VIII.—3 cases of infections due to the Staphylococcus pyogenes aureus.

Group IX.—Miscellaneous cases. Infective endocarditis, purpura, jaundice, chronic lead poisoning, chronic renal disease, coal gas poisoning, acute poisoning of unknown origin, eclampsia.

Hæm-agglutinins.

Technique.—The blood was collected for these experiments in 0·85-per-cent. pure sodium chloride, and 0·85-per-cent. pure sodium citrate in distilled water. The corpuscles were then centrifuged and thoroughly washed free from plasma in sodium chloride. A 5-per-cent. suspension of the washed corpuscles was made in normal saline. The blood was also collected in glass tubes and centrifuged in the course of an hour or so when clotting had completely taken place, as by this means a serum was obtained showing no red tinge.

In experiments on hæm-agglutinins, hæm-opsonins, and hæmo-lysins, the investigations were made immediately the blood was withdrawn.

For the agglutinative tests, one measured volume of the 5-per-cent. solution of *washed* red blood corpuscles was drawn into a capillary tube with an equal volume of blood serum. These were then thoroughly mixed and

* I am greatly indebted to one of the workers in my laboratories, Mr. H. A. F. Wilson, for much valuable help in obtaining samples of the blood from the cases which I was investigating.

incubated usually for 1 hour at 37° C. in a horizontal position in an incubator suitable for such purposes. At the expiration of this time, the contents of the capillary tube were blown out on to a slide, a glass cover-slip was placed over the mixture and the specimen was examined microscopically. In many instances, the agglutinative effect was obvious to the naked eye.

In some cases, incubation of the tubes was allowed to continue for several hours, but no advantage was gained by the extra time, and, in some instances, a hæmolytic action interfered with the results.

Experiments were also made with the centrifuged red cells undiluted with salt solution for the agglutination tests, but the results were unsatisfactory.

A definite suspension of *washed* red cells in salt solution, and a definite volume of the corpuscles and of serum were employed, so as to obtain certain essential advantages:—

(1) By employing the washed red cells, any agglutination which occurs must be due to the interaction of the red cells and of the serum added.

(2) We can add any desired serum and examine its properties, which could not be done with the unwashed red cells.

It was found that even in cases in which the agglutinative effect brought about by mixing equal volumes of serum and red cells was marked, yet if we diluted the serum with normal saline previous to the mixing with the red cells, the effect was considerably diminished, and, according to the amount of dilution, rapidly lost. This confirms the observations made some years ago by Shattock. If, however, we diluted the specific serum with an inactive serum, the results were much more satisfactory than by the method of dilution previously referred to.

Hæm-agglutination.

Shattock,* in 1899, communicated a paper to the Pathological Society of London, on chromocyte clumping in acute pneumonia and certain other diseases. His observations were carried out for the purpose of determining whether the blood serum from patients suffering from acute pneumonia, erysipelas, or acute rheumatism, had any effect upon the rouleaux formation of normal human blood. He found, by adding 1 loop of normal human blood to 1 loop of pneumonic serum, that the chromocytes ran together. On the nodal points the discs were clustered into large knotted masses. Similar observations were made in the blood from the other acute diseases just referred to. It is interesting, however, to note that in all these observations mentioned by Shattock in his communication, which was the first on this

* S. G. Shattock, "Chromocyte Clumping in Acute Pneumonia and certain other Diseases, and the Significance of the Buffy Coat in Shed Blood," 'The Journal of Pathology and Bacteriology,' 1900, vol. 6, No. 3, p. 303.

subject in human hæmatology, he always employed *normal human blood* and added to it the *immune serum*. The importance of this detail will be referred to later.

In 1900, Grünbaum* read a preliminary report which was published in the 'British Medical Journal' on the agglutination of red blood corpuscles. He came to the following conclusion as the result of his preliminary investigations: "While the serum from a case of typhoid would clump the corpuscles in the blood from another disease, it did not clump the corpuscles from the same disease. The same held good for scarlet fever."

It appears from the investigations which I have made that it is extremely common to obtain agglutination by allowing normal serum and immune red cells, or normal red cells and immune serum, to interact. In every instance a control experiment was also made by employing normal serum and normal red cells. Agglutination was not observed when normal serum was added to normal red cells, either of the same individual or from another healthy person, with one exception—a serum from an apparently healthy medical student working in my laboratory caused marked agglutination of my red cells. There was no auto-agglutination observed in this instance, and no other normal serum agglutinated either my red cells or his red cells.

The class of cases in which agglutination is most commonly met with appears to be patients suffering from tuberculosis. In this disease agglutination of human red corpuscles by one of the methods already referred to is a matter of common occurrence. In these cases examples of iso-agglutination have been obtained, and also the still rarer phenomenon of auto-agglutination. This can be very well shown in an experiment which was conducted with the red blood cells and the serum obtained from a very severe case of pulmonary tuberculosis:—

No.	Equal volumes of—	Result.
1	Immune serum (tuberculous) and normal red cells ...	Pronounced agglutination was obtained (iso-agglutination).
2	Immune serum (tuberculous) and immune red cells (tuberculous)	Similar result (auto-agglutination).
3	Immune serum (tuberculous) and immune red cells (epilepsy)	Same result (iso-agglutination).
4	Normal serum and normal red cells	No agglutination.
5	Normal serum and immune red cells (tuberculous) ...	Good agglutination (iso-agglutination).

The blood obtained from a case of chronic pulmonary tuberculosis gave a result which is commonly met with. The immune serum caused a high

* A. S. F. Grünbaum, 'British Medical Journal,' 1900, vol. 1, p. 1089.

degree of agglutination when added to normal red cells. There was no auto-agglutination, and normal serum did not react when added to the immune red cells of this case.

In another example of pulmonary tuberculosis, the immune serum did not react when mixed with normal red cells or with the red cells from the same case, but normal serum produced a high degree of agglutination when mixed with the tuberculous red cells.

The agglutinative properties from seven cases of acute pneumonia and two cases of acute pneumococcic empyæma were subjected to a detailed examination, and, as some of the results were of interest, they may be expressed concisely and briefly :—

The serum from one case of acute pneumonia when added to the red cells from the same case gave no result, but when added to normal red cells a high degree of agglutination occurred. When this pneumococcic serum was diluted with salt solution and added to normal red cells, employing dilutions of 1 : 20 and 1 : 200, no agglutination took place.

Another case of acute pneumonia gave results of similar interest. The blood was obtained from a patient suffering from acute pneumonia just after the crisis. The immune red cells and the immune serum when mixed, failed to react, but when the immune red cells were mixed with normal serum, or when the immune serum was added to normal red cells, a high degree of agglutination resulted. The serum from this case was also mixed with the patient's unwashed red cells ; the result was similar to that obtained with the washed red cells. The immune serum from the second case of acute pneumococcic empyæma produced very slight agglutination when mixed with the immune red cells. Normal serum gave a similar result with these red cells, but the immune serum in the presence of normal red cells, and of the red cells from another case of acute pneumococcic empyæma, produced considerable agglutination ; in fact, the clumps reached an enormous size, and there were very few free red cells present.

In the blood obtained from a case of long standing epilepsy, a striking instance of auto-agglutination was observed. The patient's serum, when added to his own red cells, caused a high degree of agglutination. The same effect was observed when the immune serum was added to normal red blood corpuscles. In this specimen of blood we have one of the few examples, and yet one of the most striking instances of auto-agglutination.

In another case of long standing epilepsy the immune serum failed to react in the presence of the immune red cells or normal red cells, but when normal serum was mixed with the immune red cells, they collected together into large and tight clumps.

Specific Hæm-agglutinins.

In these experiments, if a serum was found to produce marked agglutination when mixed with certain red blood corpuscles in the manner described, it was tested for the presence of specific agglutinins. A definite measured volume of the immune serum was mixed with a similar volume of a thick deposit of the washed red cells obtained at the end of the last stage of centrifugalisation. These red cells were not suspended in saline, so that the serum could be more intimately mixed with a dense volume of red cells, and would not be diluted with salt solution. The mixture was incubated in sealed capillary tubes for several hours at 37° C. It was then centrifuged at high speed, and the clear serum tested on a 5-per-cent. suspension of washed red cells as before.

The first case in which the specific effect may be referred to is one of great interest. The immune serum from a case of acute pulmonary tuberculosis was found to produce a high degree of agglutination when mixed with the red blood corpuscles obtained from a case of anesthetic leprosy, and with normal red cells, but there was absence of any auto-agglutination. The serum from this case was "saturated" with the red blood corpuscles (leprosy) in the manner referred to in the technique. The mixture, after incubation, was centrifuged, and the clear fluid which separated was added to the leprosy red cells and to normal red cells. No agglutination resulted. When the immune serum was saturated in a similar manner with the normal red cells, the clear fluid obtained did not agglutinate normal red cells, and only slight agglutination occurred when it was added to the leprosy red cells. The serum from a case of chronic surgical tuberculosis which had been found to produce a high degree of agglutination when added to normal red cells and the leprous red cells, although it failed to show any auto-agglutination, was saturated with normal red cells. The clear fluid was then added to normal red cells, but no agglutination resulted. When added, however, to the leprous red cells, distinct agglutination occurred. From these experiments, it would seem that the leprous red cells had the power of removing entirely the agglutinative properties of the serum from the case of acute pulmonary tuberculosis; this serum, as the result of saturation, failing to agglutinate either the leprous red cells or the normal red cells. When, however, the serum from the case of acute pulmonary tuberculosis, and from the case of surgical tuberculosis, was saturated with normal red cells, the result was to abolish the clumping action with the normal red blood corpuscles, but to leave a certain degree of agglutination for the red cells of the leper.

The simple agglutinative properties of the blood from the case of acute

pneumonia, on which these saturation experiments were made, have been referred to already in detail. The immune serum, when added to normal red cells, caused a high degree of agglutination. When saturation was completed, it was found that the resulting clear fluid failed to agglutinate normal red cells, but still had the power of agglutinating the red cells from a case of epilepsy, although to a less extent than previous to saturation with normal red cells. Normal serum and the pneumonic red cells, when mixed together, caused a high degree of clumping to occur. The clear fluid which resulted from the saturation of this sample of immune red cells and normal serum would not react with the immune red cells, with which excessive clumping had occurred previous to saturation.

The serum from another case of acute pneumonia, just after the crisis, failed to react with the immune red cells or with normal red cells, but normal serum, when added to these immune red cells, produced a high degree of clumping. The examination of the blood in this instance is, therefore, of considerable interest, because the immune serum had no agglutinative effect on either immune or normal red cells, but normal serum which had no clumping action on normal red cells and on certain other examples of immune red cells, yet produced enormous clumping in the presence of the pneumonic red cells. When normal serum was saturated with these immune red cells, the clear fluid that resulted failed to agglutinate them, but produced some degree of agglutination in the presence of a sample of tuberculous red cells which had given considerable clumping with the normal serum previous to agglutination. When the normal serum and tuberculous red cells were mixed together and saturation was completed, the clear fluid obtained would not agglutinate the pneumococcal red cells referred to above or the tuberculous red cells. The immune serum from a case of severe pernicious anæmia and the same specimen of tuberculous red cells showed considerable clumping when brought into contact. When saturation was completed, the resulting clear fluid failed to agglutinate either the tuberculous red cells or the pneumonic red cells which had run together in large masses in the presence of the serum from this case of pernicious anæmia previous to saturation. The last series of experiments completed in this group of specific agglutinative tests are of importance, because they illustrate the fact that saturation of the pernicious anæmia serum with normal red cells, with which it failed to react previous to saturation, had no power to remove the agglutinative property from this sample of immune serum either for the tuberculous or the pneumonic red cells. In this special instance the agglutination was as well marked after as before saturation.

Bacterial- and Hæm-agglutinins present in the same Serum.

The serum obtained from a case of typhoid infection was found to possess a very high degree of agglutination on normal red cells, but did not possess any auto-agglutinative properties. When the patient's serum was saturated with his own red cells, it was found that it still possessed as high a degree of agglutination on typhoid bacilli as before saturation. The same serum when saturated with normal red cells, on which it had a marked agglutinative action, still possessed the same power of clumping typhoid bacilli as in the previous experiments. A further experiment was performed with the serum from another case of typhoid fever. Saturation of this serum, which had caused marked agglutination when added to typhoid bacilli and normal red blood corpuscles, removed the agglutinative action on the red cells, yet it had no effect on the clumping action of the bacilli.*

The Relationship between Rouleaux Formation and Agglutination of the Red Blood Corpuscles.

In normal blood rouleaux formation is a constant factor. In certain acute and chronic diseases the rouleaux formation is either present to a slight degree or is absent. In those cases in which its absence is noted, it may be found that agglutination of the red cells is present instead. It will be seen, however, from previous statements, that auto-agglutination is of rare occurrence, while iso-agglutination of one type or other is extremely common. It is, therefore, not strictly accurate to state that agglutination of the red blood corpuscles in certain diseases replaces or has replaced the rouleaux formation which was present in that same blood in health, because rouleaux formation, as we see it, is a true auto-effect—the red cells and the plasma from the same case. In agglutination it is generally otherwise, that is to say, the clumping of normal red cells in the presence of immune serum or *vice versâ* is an iso-effect. True agglutination, such as may be seen in the experiments which I have referred to, is never to be found in blood obtained directly from the patient. Agglutination of the rouleaux may take place in pathological blood; in some diseases it is very obvious, but here again the small clumps of rouleaux do not resemble actual agglutination of the red blood corpuscles. In a paper on acute lymphocythæmia, which I published a few years back in the 'Transactions of the Pathological Society of London,' attention was drawn to agglutination of the red cells in the fresh blood

* In this communication only a few of the experiments on specific and simple hæm-agglutinins have been referred to, as it would be unnecessary repetition to cite them all.

examined in the last stages of the disease. This, however, is not an example of true agglutination, but it is clumping of short rouleaux. Shattock noted, as already stated, that if we diluted human serum with saline, the red blood corpuscles did not run into rouleaux when mixed with this diluted serum, as in the case of the pure serum. A similar effect is the rule in the case of agglutination. In this respect, rouleaux formation and hæm-agglutination have points in common. If the blood is examined, *e.g.*, from a case of pulmonary tuberculosis in which the rouleaux formation observed in the fresh film is well-nigh perfect and free from agglutination, when this serum is mixed with normal red cells it gives a striking appearance to the blood. No rouleaux formation can be observed, but the red cells are collected into large and tight clumps, while the shape of the cells is considerably altered from the large red circular disc to small retracted bodies apparently less than half the size of the original cell, highly refractile and resembling an oil droplet. This is probably a physical action and may be dependent upon the salt contents of the serum and cells.

From what has been stated in this communication, and from very numerous unrelated observations, rouleaux formation and haem-agglutination must be regarded as distinct phenomena.

Hæm-opsonins.

Technique.—The white cells for these experiments were obtained by the method which is usually adopted for such investigations on phagocytosis. In every instance normal leucocytes were employed, but in some instances washed immune cells were obtained from various cases, and the results compared with those obtained with the normal cells. It was found, however, that practically the same result occurred whether the immune cell or the normal cell was employed for this special purpose.

A 5-per-cent. suspension of washed red blood corpuscles in normal saline (0·85 per cent.) was used in all these experiments. One volume of normal or immune leucocytes was drawn up into a capillary tube with an equal volume of washed red cells and blood serum. These were carefully mixed, sealed in a glass tube, and incubated in the usual manner. Various times were used for the incubation, varying from 15 minutes to several hours, but there was no advantage, while in many cases a disadvantage occurred in incubating for periods of longer time than half an hour.

In many instances the serum, before it was drawn up into the capillary tube, was diluted to a definite strength either with normal salt solution or with a standard normal serum. At the end of the incubation period the contents of the capillary tube were blown out on to a glass slide, and thin film

preparations were made and stained with Leishman's stain. It is most important that the film preparations should be thin and rapidly dried, as otherwise the leucocytes retract owing to imperfect fixation, and the estimation of the phagocytosis becomes impossible.

In some instances, the serum was heated to 55° C. for 20 minutes, and the phagocytic properties of the serum were then compared with those obtained with the unheated.

It will be understood from the remarks which have already been given on this subject of phagocytosis of red blood corpuscles, that numerous methods were employed and comparisons made so as to ascertain the manner in which phagocytosis was most marked. It is unnecessary to refer at great length to the very large number of experiments made, as it was only in a few instances that phagocytosis was pronounced. In quite a number of instances, whether the serum was unheated, or diluted, or heated, or whether normal or immune leucocytes were employed, the degree of phagocytosis was infinitesimal. In every instance 50 or 100 leucocytes were counted, and the number of corpuscles engulfed was carefully noted. In the large majority of cases, out of 50 cells only three or four would be found to be phagocytic. There was only one instance out of the total number investigated in which the phagocytosis of red blood corpuscles was a striking feature. This occurred in a case of jaundice, unfortunately of unknown origin. The immune red cells of the patient in the presence of the unheated undiluted immune serum from the same case and normal leucocytes gave a high degree of phagocytosis: 56 per cent. of the cells were phagocytic, and there were 33 red blood corpuscles in the 50 cells. When normal red cells were used in conjunction with the same serum and normal leucocytes, as in the previous experiment, 76 per cent. of the leucocytes were phagocytic, and 50 of these contained 46 red blood corpuscles.

In both these experiments, the serum, the leucocytes, and the red blood corpuscles were incubated in the capillary tubes in equal volumes for a period of 15 minutes at 37° C. The red cells stained well with eosin, showed no degenerative changes, and none of the leucocytes contained definite "Ghosts." This serum was found to possess a high agglutinative property for normal red cells, but it was not tested on the immune red cells. It was found to be free from any hæmolytic action, either on the patient's red cells or on normal red cells.

As previously mentioned, *these were the only two examples* out of some 250 experiments made in which *over* 20 per cent. of the leucocytes were phagocytic. The other instances in which the phagocytosis was sufficiently pronounced to record were, firstly, a case of chlorosis, in which 20 per cent.

of the leucocytes contained red blood corpuscles, the phagocytic mixture consisting of the immune red cells, normal serum, and normal leucocytes. In the same experiment, conducted with immune serum, only three cells were phagocytic.

The next case was an example of severe anæmia, secondary to hæmorrhage, in which 16 per cent. of the leucocytes were phagocytic. In this experiment, as in the last, the higher degree of phagocytosis occurred by the interaction of normal serum and immune red cells.

In a case of acute peritonitis and appendicitis, 20 per cent. of the leucocytes were phagocytic, but in this experiment the higher degree of phagocytosis occurred by the interaction of immune serum and immune red cells, in conjunction with normal leucocytes.

In all the other experiments it was often found that 5 to 10 per cent. of the leucocytes contained one red blood corpuscle, while in many instances there was no phagocytosis present.

It has been suggested that one of the main causes of anæmia is due to the presence in the patient's serum of a certain substance which acts upon the red blood corpuscles, which in turn became devoured by the leucocytes. There is nothing in these experiments to support this view. In those cases in which the anæmia was most intense, the phagocytosis was present to an infinitesimal degree, while in the cases previously referred to in which the phagocytosis of the red blood corpuscles was such a striking feature, the anæmia was quite of a mild type.

As I have already stated, there was no relation in these experiments between the extent of the agglutinative, hæmolysing, and opsonic properties of the same serum. A very large number of these immune sera had a more or less high degree of agglutinative action, yet the degree of hæmolysis which they were capable of producing was only occasionally striking, and the opsonic property was the least marked. Barratt,* in his experiments, conducted with the blood of the lower animals, has shown that even with unheated immune serum, phagocytosis of red blood cells can occur without the serum having either hæmolytic or agglutinative properties, and he favours the view that the phagocytosis is induced by some body which can be placed amongst the order of opsonins. R. D. Keith,† in a paper also published in the 'Proceedings of the Royal Society,' from work done in the laboratories of the London Hospital, came to the following conclusions as the

* Barratt, "The Phagocytosis of Red Blood Corpuscles," 'Roy. Soc. Proc.,' 1905, vol. 76, Ser. B, p. 524.

† R. D. Keith, "On the Relationship between Hæmolysis and the Phagocytosis of Red Blood Cells," 'Roy. Soc. Proc.,' 1906, vol. 77, Ser. B, p. 537.

result of some interesting experiments:—"That the substance which induces phagocytosis is partially destroyed by heat, while the hæmolytic amboceptor is entirely thermo-stable." And secondly: "The hæmolytic amboceptor may be present in considerable amount in a hæmolytic serum without inducing phagocytosis, notwithstanding prolonged contact of the amboceptor with the red blood cells."

The experiments referred to in this communication entirely agree with the observations of Barratt and Keith, conducted with the blood sera and cells from the lower animals. There was nothing to show that the agglutinative, opsonic, or hæmolysing properties of normal or immune sera on red blood corpuscles have any direct relation to one another.

Hæmo-lysins.

Technique.—A 5-per-cent. suspension of washed red cells in 0·85-per-cent. saline in distilled water was employed for these experiments. Normal red cells were allowed to act in the presence of normal serum and immune serum, immune red cells in normal serum, immune red cells in immune serum, and normal and immune red cells in immune serum, to which a definite volume of native and foreign complement had been added.

The serum to be tested was always carefully noted to be free from any blood tinging.

The mixture of red blood corpuscles, serum, and salt solution was incubated, usually for two hours, immersed in sealed glass tubes in water at 37° C., and placed in an incubator at the same temperature. In some cases the incubation period was considerably extended, but two hours was found to be sufficient. At the end of that time the tubes were put in the ice-safe and examined at the end of about 12 hours. The actual dilutions which were employed were as follows:—The capillary tubes were carefully graduated, and in each tube, in every experiment, the total contents amounted to four volumes, of which one volume of washed red cells was a constant quantity, and the amounts of serum and salt solution were in inverse ratio; the first tube contained one volume of washed red cells and three volumes of serum, without any salt solution, while the tenth tube in each series contained 2·5 volumes of salt solution and 0·005 volume of serum.

It should be noted that the strength of the salt solution employed for these experiments was 0·85 or 0·9 per cent., but it was found in these investigations that certain red cells underwent some degree of hæmolysis when presented to a 0·9-per-cent. salt solution. This is a most important fact, because it shows that physiological salt solution has a power of hæmolysing immune human red cells under certain conditions. This special

instance occurred in the case of a girl suffering from acute poisoning of unknown origin. Here, although immune serum failed to hæmolyse the immune red cell, yet physiological salt solution produced slight hæmolysis when presented in the same amount as the serum in the hæmolytic mixture, while, when present greatly in excess of the serum, marked hæmolysis occurred.

In all these experiments it was only occasionally that the hæmolytic action was distinctly shown ; in the majority of instances no hæmolysis occurred.

In *Group I*—cases of pneumonia—there were two instances in which hæmolysis occurred. The first example was a case of acute pneumonia, just before the crisis, in which a mixture containing 75 per cent. of auto-immune serum produced complete hæmolysis on auto-immune red cells. The hæmolytic action occurred down to a dilution in which the serum was present to the extent of 25 per cent.

The second example was a case of acute pneumonia, just after the crisis, and in this instance the immune serum hæmolysed normal red cells, but failed to hæmolyse the auto-immune red cells. Well-marked hæmolysis occurred with 75 per cent. of serum. On diminishing the amount of serum, the effect became less until 37·5 per cent. of serum caused only a very slight hæmolytic action.

In *Group II*—cases of tuberculosis—hæmolysis was only occasionally met with. In each instance it was the action of the immune serum obtained from cases of acute pulmonary tuberculosis on normal red cells. In these experiments the hæmolysis was well marked, while auto-hæmolysis did not occur.

In *Group VI*—pernicious anæmia. All the cases referred to under this heading were well-marked examples of the disease. It may be of interest to mention that out of the several cases which were examined, in every instance the serum was either bile-tinged or more frequently of a canary-yellow colour, due to the presence of a pigment which failed to give any spectrum, and which was not bile-pigment. In most of these cases neither auto- nor iso-hæmolysis occurred.

In one case extremely slight hæmolysis occurred by the action of immune serum on normal red cells, while there was no auto-hæmolysis.

In another case there was no hæmolysis during the patient's lifetime, but the serum obtained at the *post-mortem* examination, which contained bile-pigment, was found to have a very strong hæmolytic action on normal red cells; 75 per cent. of serum almost completely hæmolysed the red blood corpuscles, while 12·5 per cent. of serum produced distinct but slight hæmolysis. It was found that in each tube in which hæmolysis occurred, clumps of streptococci were present. This streptococcus, which was obtained in pure

culture from the hæmolytic tubes and from the blood at the *post-mortem* examination, was an atypical streptococcus, according to the classification of Andrewes and Horder. The micro-organism itself had also the power of exciting hæmolysis, so that the hæmolytic action which was found to take place in this sample of immune serum was not due to the serum itself, but to the streptococci which it contained in such large numbers.

The third example was a severe case of this disease in which there was no auto-hæmolysis, although the blood was examined on several occasions, and there was no auto-hæmolysis even after the immune serum had been complemented, but a high degree of hæmolysis occurred by allowing the immune serum to act on the red blood corpuscles from a case of acute lobar pneumonia; 75 per cent. of serum produced complete hæmolysis; 62·5 per cent. and 50 per cent. of serum produced incomplete hæmolysis; 37·5 per cent. slight, and 25 per cent. very slight, hæmolysis.

In a case of very severe anæmia, due to hæmorrhage, there was no hæmolysis, but the immune serum had the power to a slight degree of hæmolysing normal red cells. The second case was an example of acute lymphocythæmia in which there was no auto-hæmolysis, but a slight hæmolytic action of the immune serum on normal red cells.

Group VII.—In the streptococcal cases hæmolysis occurred in two instances. The first was a case of streptococcal pyæmia due to an atypical streptococcus, in which the immune serum produced well-marked hæmolysis on normal red cells down to a dilution of 25 per cent. of serum, while there was no auto-hæmolysis. The second case was an example of acute erysipelas due to the *Streptococcus pyogenes*, in which only very slight hæmolysis occurred in the presence of 75 per cent. of serum. Here, again, the interaction was limited to the immune serum and the normal red cells.

Group IX.—In the case of chronic lead poisoning there was no auto-hæmolysis, but the immune serum had a slight but distinct hæmolytic action on normal red cells.

In a case of jaundice, probably carcinomatous, there was distinct auto-hæmolysis with 75 per cent., 62·5 per cent., and 50 per cent. of serum. This case, however, was the only example in the whole series of experiments in which there was an auto-hæmolytic action. If this case proved to be carcinomatous, it would be the only example of this disease in the whole series investigated.

Conclusions.

It will be noticed that a striking feature, with one exception, in all these experiments is the absence of the auto-hæmolytic action and the hæmolysing property of the same serum on normal red blood corpuscles.